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Methods of Screening Blueberry Seedling Populations for Drought Resistance

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Abstract. **Two methods of evaluating seedling drought resistance in *Vaccinium* (blueberry) spp. were examined. Twenty interspecific populations were greenhouse-grown and either matric-stressed in a dry 1 sand : 1 soil medium or osmotic-stressed in a nutrient solution containing polyethylene glycol (PEG). In both tests, population means were separated statistically by shoot damage ratings. The correlation (r = 0.46) between the two tests was positive and significant. Progenies of clones JU64 and JU62, which are sister seedlings (*V. myrsinites* Lamark x *V. angustifolum* Aiton), were the most drought-resistant. The soil screening test appeared more accurate because it grouped populations with common parentage. These tests indicated that the progenies differ in genetic capacity to resist drought.**

The supply of water for use in agriculture is becoming increasingly limited. Trickle irrigation reduces moisture loss through evaporation and run-off, but even this conservation practice may not be adequate in dry years. An efficient manner of maintaining high productivity while conserving water is by growing drought-resistant plants.

Genetic variability for drought resistance is probably present in *Vaccinium* (subgenus *Cyanococcus*), since some species grow over a wide range of soil and climatic conditions. Some species can be found growing on nutrient impoverished sands that are subjected to frequent drought, whereas other tolerate poorly drained acidic soils that are high in organic matter (2, 4). In order to identify drought-resistant genotypes for use in breeding, an effective screening test is required. The objective of this study was to evaluate two greenhouse methods of screening for seedling drought resistance in blueberry.

The methods involved growing seedlings in either a) a nutrient solution containing PEG, which produces an osmotic stress (PEG test), or b) a sand : soil mix that was allowed to dry to produce a matric stress (soil test). The two tests were conducted in the same greenhouse on adjacent benches. Each test was arranged in a randomized complete block design, and seedlings from the same 20 populations were screened. Each of four blocks consisted of 15 individuals from each population. Three replicates of five plants were randomly placed in each block. The parents of the seedlings used in this study are presented in Table 1. Both methods were evaluated by a shoot damage rating scale based on percent leaf necrosis, leaf drop, and stem dieback (Table 2).

Seeds were germinated in March, and the seedlings used in the test were transplanted in May to 20.3 × 61 cm plastic flats containing peat–vermiculite mix (Jiffy mix). The seedlings were grown in a greenhouse and given normal plant care. In mid-August, the roots of the seedlings were rinsed and either placed in a tank containing nutrient solution (PEG test) or in a bed of sand : soil mix (soil test).

In the PEG test, seedlings were placed in five 57 × 127 × 9 cm black plastic tanks covered with a 1.9-cm-thick styrofoam lid that floated on the surface of the nutrient solution. One tank served as a control and received only nutrient solution. Each tank held 50 liters of solution and 300 equally spaced seedlings. A pump was used to aerate the solution. The nutrient solution described by Erb et al. (3) was used. Fresh nutrient solution was added once a week. The seedlings were grown for 2 weeks in the nutrient solution before the PEG (Carbowax 4000, M_r = 3500 to 3700) was added. The PEG was added in increments of 20 g·liter^{-1}, approximating an osmotic potential of −0.05 MPa (5), for 5 days then given 2 days to equilibrate. This procedure was repeated for another week. At the end of 2 weeks, the osmotic potential of the solutions was at about −0.5 MPa. The plants were left in this −0.5 MPa solution for 17 days. The percentage of seedlings that were severely injured was determined after the first and second week of exposure to −0.5 MPa and at the end of the experiment. Plants scoring a shoot damage rating of 4 or less were considered severely injured (see Table 2).

In the soil test, a greenhouse bench 823 × 137 × 16 cm was filled with a mixture of 1 sand : 1 soil (v/v) that had a pH of 5.7 and a texture of 75% sand, 15% silt, and 10% clay. The bench was divided equally into four blocks, and six tensiometers were equally spaced throughout the bench at a depth of 14 cm. The seedlings were planted 5 cm within and 10 cm between rows and were allowed 5 weeks of adjustment before water was withheld. Plants were stressed in stages to approximate the slow drying that occurs in the field. Seedlings were subjected to five stress levels: 1) Dry to 0.06 MPa, 2) dry to 0.08–0.09 MPa, 3) 3 days at 0.08-0.09 MPa, 4) 5 days at 0.08–0.09 MPa, and 5) 7 days at 0.08–0.09 MPa. After each stress level, the bed was saturated and the number of plants severely injured determined.

In the soil test, each block was fertilized with 36 g of Peters acid special 21N–3.1P–5.8K fertilizer in 4.5 liters of water once a week starting with the end of the second week in the bed. Everyother week 0.22 g·liter^{-1} (0.03g Fe/liter) of ethylenedinitrilo-tetraacetic acid, iron(III) derivative, and sodium salt was added to the mixture. Once a month, 6.22 g·liter^{-1} of Miller (VHPF) complete fertilizer was added with the Peters acid special. The following is the analysis in g·liter^{-1} of the complete fertilizer: 0.37 N, 0.68 P, 0.77 K, 0.35 Ca, 0.004 B, 0.008 Cu, 0.006 Fe, 0.007 Mn, 0.003 Mo, 0.006 Zn, and 0.009 Mg. While the plants were being stressed, they were fertilized after each drying cycle.

To determine when to terminate each test, the 20 seedling populations were rated for percent severely injured per week in the PEG

Table 1. Parentage of blueberry clones used as parents to produce progenies screened for drought resistance.

Clone	Ploidy	Species complement
JU10 & JU11	6×	Tifblue (*Vaccinium ashei* Reade) x US41 (*V. atrococcum* Heller)
JU62 & JU64	4×	*V. myrsinites* Lamark x *V. angustifolium* Aiton
US75	4×	Fla-4B (*V. darrowi* Camp) x Bluecrop (*V. corymbosum* L.)
US79	5×	Fla-4B x (*V. constablaei* Gray x *V. ashei*)
G111	4×	*V. corymbosum*
US226	4×	*V. myrtilloides* Michaux x *V. atrococcum*
G362	4×	*V. corymbosum*

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Table 2. Shoot damage rating scale used to determine drought resistance in blueberry seedlings screened in a nutrient solution containing polyethylene glycol or in a dry soil bed.

Score	Description
9	= Healthy
8	= 1% to 10% leaf necrosis and one to three leaves dropped.
7	= 11% to 20% leaf necrosis and two to five leaves dropped.
6	= 21% to 35% leaf necrosis and as much as 25% leaf drop.
5	= 36% to 50% leaf necrosis and as much as 50% leaf drop.
4	= 51% to 75% leaf necrosis and as much as 80% leaf drop.
3	= 76% to 100% leaf necrosis and as much as 100% leaf drop, but < 25% stem dieback.
2	= 100% leaf drop and > 25% stem dieback.
1	= Stem is dead.

Table 3. Percent blueberry seedlings scored severely injured per week (PEG test) and per stress level (soil test) in two different drought screening tests.[z]

Test	Severely injured (%)
PEG	
Week 1	36.0
Week 2	44.6
Week 3	50.0
Soil	
Stress level 1	0.4
2	2.4
3	25.9
4	46.8
5	63.3

[z]A plant was considered severely injuried if it scored 1 through 4 in the shoot damage rating scale (see Table 2).

test and per stress period in the soil test (Table 3). The PEG test was terminated when 50% of all the seedlings were rated as severely injured. After the fifth stress period, 63.3% of the seedlings were rated severely injured. Thirty-one percent of the plants in the PEG test and 24% of those in the soil test received shoot damage rating scores of 7 or above and were considered drought-resistant. In both tests, considerable damage occurred to the plants. The majority of the selected plants from the PEG test did not survive transplanting into soil.

The PEG and soil tests were significantly correlated ($r = 0.43$; $P = 0.0001$) when stress period 4 and PEG week 3 were compared, and $r = 0.46$ ($P = 0.0001$) when stress period 5 and PEG week 3 were compared. The mean shoot damage rating scores of screening tests for week 3 of the PEG test and stress period 5 of the soil test are presented in Table 4. In both tests, the populations differed significantly in mean shoot damage rating, ranging from 6.73 to 2.51 in the soil test and 5.81 to 2.80 in the PEG test. The progeny of US79 x G362 scored highest in the PEG test, but was intermediate in the soil test. In the soil test, the progeny of JU64 x JU62 and the populations with either JU64 or JU62 as a parent ranked highest in drought resistance. JU64 and JU62 are sister seedlings; one of their parents is a southern blueberry species found growing in sandy areas subjected to frequent drought (*V. myrsinites*) (4, 6). The other parent is a northern blueberry species found on rocky uplands, dry sandy areas, and swamp borders (*V. angustifolium*) (4). The PEG test did not separate some of the JU64 and JU62 populations, but did separate the population of JU62 x G362 as being significantly less drought-resistant than the other JU64 and JU62 populations. The populations of G362 and G111 crossed with US226 ranked at the bottom in the soil test. One of the parents of US226 is a selection of *V. atrococcum* Heller, a species that is found primarily in moist habitats (94). Populations of G362 and G111 (both *V. corymbosum* L.) and all crosses of US75 scored intermediate in drought resistance. *V. corymbosum* is a species generally found in moist environments. One parent of US75 is a selection of *V. darrowi* Camp, a species native to southern United States, which can be found growing in sandy areas that are exposed to frequent water deficits (4, 6).

The stress imposed in the two tests differed. In the PEG test, the addition of PEG produces an osmotic stress that is assumed to mimic a matric stress, the most important component of water potential in a dry soil. PEG has been used by researchers (1, 5) because when applied to a nutrient solution a uniform osmotic stress is produced. The molecular weight of PEG (3500 to 3700) used may have been taken up by the roots of some of the seedlings, producing toxic effects, and may have resulted in the poor transplant survival rate of PEG-selected plants. Another difference between the tests was the higher salt concentration of the nutrient solution that continually bathed seedling roots. This continued exposure may have allowed more osmotic adjustment to occur in the leaves of drought-susceptible plants than usually occurs in a field situation (7).

It appears that the interspecific seedling populations varied in genetic capacity to resist drought and that the soil screening test accurately identified drought-resistant blueberry seedlings. The soil test appeared more accurate than the nutrient solution test because it grouped populations of common parentage.

Table 4. Mean shoot damage scores for 20 blueberry progenies screened for drought resistance in either a nutrient solution containing polyethylene glycol (PEG test) or a bed of dry soil (soil test).

PEG test			Soil test		
	Shoot rating[z]			Shoot rating	
Progeny	Mean	N[y]	Progeny	Mean	N
US79 x G362	5.81 a[x]	60	JU64 x JU62	6.73 a	60
G111 x JU64	5.67 ab	60	JU62 x G362	5.80 ab	60
G362 x JU62	5.46 ab	60	G362 x JU62	5.23 a–c	60
JU64 x JU62	5.18 a–c	60	G111 x JU64	5.23 a–c	60
G362 x G111	5.02 a–d	60	G362 x JU64	5.17 a–d	60
G362 x US75	4.70 a–e	60	G111 x US75	4.28 b–e	60
JU10 x G362	4.64 b–e	45	US226 x US75	4.25 b–e	60
G362 x JU64	4.64 b–e	60	JU10 x G362	4.11 b–e	45
G111 x US75	4.58 b–f	60	JU11 selfed	3.88 b–e	60
US226 x G362	4.54 b–f	60	US79 x G362	3.69 c–e	60
US75 x G362	4.21 c–g	30	G111 x G362	3.65 c–e	60
US226 x US75	4.19 c–g	60	G362 x JU10	3.42 c–e	45
JU11 selfed	4.10 c–g	60	G362 x US75	3.35 c–e	60
G111 x G362	3.88 d–h	60	US75 x G362	3.14 de	30
G362 x JU10	3.58 e–h	45	G362 x G111	2.79 e	60
US226 x G111	3.52 e–h	60	G362 x US226	2.95 e	60
JU62 x G362	3.42 f–h	60	US226 x G111	2.90 e	60
JU10 x JU11	3.55 gh	60	US226 x G362	2.83 e	60
G362 selfed	3.34 gh	60	JU10 x JU11	2.61 e	60
G362 x US226	2.08 h	60	G362 selfed	2.51 e	60

[z]Shoot damage rating scale is presented in Table 2.
[y]Number of seedlings screened per progeny.
[x]Mean separation by Duncan's multiple range test, 5% level.

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Comparison of Two Nitrogen Fertilizer Sources for Highbush Blueberries

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Abstract. **Highbush blueberries (*Vaccinium corymbosum* L.) were fertilized with equal amounts of nitrogen from ammonium sulfate (AMS) or sulfur-coated urea (SCU) for 6 years. The plots treated with SCU yielded significantly more than the AMS plots three out of the five harvest years. Except for plant size in 1984, treatments did not significantly affect berry size, plant survival, and other vegetative characteristics. Soil pH after 5 years was significantly lower on the AMS treatments than on the SCU-treated plots (5.4 vs. 5.7). The nutrient content of the leaf tissue did not differ significantly by fertilizer source. At the rate used in this study, SCU would be an acceptable nitrogen source for blueberries compared to AMS, but SCU is less effective in maintaining low soil pH.**

Optimum highbush blueberry production requires the maintenance of a lower soil pH than commonly occurs in many upland soils. Because of this management constraint, ammonium sulfate (AMS), an acid-forming nitrogen source, commonly is recommended for blueberry production (2, 7). AMS is not readily available in this area. Therefore, an alternate acid-forming nitrogen source would be desirable. The Tennessee Valley Authority (TVA) was evaluating the use of sulfur-coated urea (SCU) as a nitrogen source for a variety of crops. SCU could become readily available. The purpose of this study was to compare the effects of SCU and AMS on blueberry growth, yield, soil pH, and nutrient availability.

One-year-old plants of eight highbush blueberry cultivars (Berkley, Bluecrop, Blueray, Collins, Darrow, Earliblue, Jersey, and Patriot) were established in May 1979 on a Crider silt loam (Typic Paleudalf, fine-silty, mixed mesic) at the Univ. of Kentucky College of Agriculture Research and Education Center, Princeton. Prior to planting, the soil was fertilized based on a soil test and amended with finely ground elemental sulfur (95% passed through a 325-mesh screen) to adjust the pH to 4.5 (2, 4). Prior to planting, 3.8 liters of wet peatmoss was incorporated in each planting hole. Plants were spaced 1.2 m apart with 3.0 m between rows, about 2700 plants/ha. A fresh hardwood sawdust mulch was applied annually to renew the mulch to a depth of 10 cm, and the planting was irrigated by trickle irrigation as needed. Finely ground elemental sulfur was applied to all plots at the rate of 167 $g \cdot m^{-2}$ in Winter 1983. This treatment reduced the soil pH of the planting from 6.1 in 1983 to 5.4 in 1984.

Each plot consisted of one cultivar. Eight four-plant plots per block were split in half to form two two-plant subplots per plot, in each of two blocks, for a total of 32 subplots. The two fertilizer treatments were 92 $kg \cdot ha^{-1} \cdot year^{-1}$ of N as AMS (20% N) or SCU (36.1% N). Each fertilizer was broadcast in two equal applications—the first week of May (at bloom) and 6 weeks later in June. Treatments were started in 1980, and the N rates were increased to 138 and 207 $kg \cdot ha^{-1} \cdot year^{-1}$ in 1983 and 1985, respectively. These adjustments were made to maintain optimum foliar nitrogen levels (5) and were in accordance with commercial recommendations (2, 7). Applications of P and K were not made in this study.

Fruit were hand-harvested beginning with the 1981 season, and the yield per plant was recorded. All vegetative growth measurements were made during the fall/winter season following the summer harvests. The number of new canes arising within the area from the mulch surface to 20 cm above it was recorded. A growth index was calculated annually by dividing the sum of the maximum height plus maximum width by 2 (3). In 1985, the average weight per berry was calculated based on a 25-fruit harvest. Soil and leaf samples were collected from each treatment-replication combination on 22 July 1985. Soil and leaf samples collected in previous years were used solely to monitor the general soil and nutritional status of the planting and were inappropriate for statistical analysis. The means of each two-plant subplot for each variable (except percent survival) were analyzed statistically using the SAS GLM procedure. Treatment effects on plant survival were analyzed by collapsing across replications and cultivars and using the χ^2 test.

Fertilizer source had a significant effect on yield 3 out of 5 years (Table 1). In every year, yield for plants receiving SCU was as great as or greater than those receiving AMS. Berry size in 1985 averaged 1.7 g/berry for both fertilizer treatments. Plant survival at the end of 1985 (69.4% and 75.0% for SCU and AMS, respectively) was also not significantly different for the two treatments. Top growth and number of 1-year-old canes for SCU-treated plants were as high as or higher than the AMS-treated ones (Table 1). Plants treated with SCU in 1984 were significantly larger than those treated with AMS. No significant differences between the two fertilizer treatments were observed in soil and leaf nutrient levels (Table 2). However, the pH was higher in soil fertilized with SCU than that treated with AMS.

The SCU treatment resulted in a 34%, 31%, 74%, 60%, and 13% greater yield than those receiving AMS in 1981 through 1985, respectively. The greatest difference in yield

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